GLASGOW

Glandular and Locomotor Structures and Functions In the Larva of Phytonomus Punctatus Fab

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GLANDULAR AND LOCOMOTOR STRUCTURES AND FUNCTIONS IN THE LARVA OF PHYTONOMUS PUNCTATUS FAB.

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Introduction.

This paper presents the results of a series of studies upon the peculiar method of cocoon-building, and the secretion of silk by the larva of the clover leaf weevil, *Phytonomus punctatus* Fabr., in which was encountered a most unusual alternation of glandular functions. Some notes on the locomotion of this larva are also given.

Materials and Methods.

The *Clover Leaf Beetle*.

Phytonomus (*Hypera*) *punctatus*, Fabricius.

Order Coleoptera; Family Curculionidae.

This beetle, which sometimes becomes a serious clover pest, was introduced into America from Europe some time before the middle of the last century. Its presence on this continent was first reported when Dr. Le Conte received a single specimen from Canada and another specimen from Pennsylvania. In 1881 it caused great damage to clover fields in Yates Co., New York, and since then has become widely distributed.

Both larva and beetle feed upon the leaves of clover, eating indifferently *Trifolium pratense*, *Trifolium repens*, or *Trifolium hybridum*, and also attacking *Melilotus*. This insect passes the winter partly in the egg stage and partly as small larvae; and occasionally a beetle also hibernates successfully. The larvae feed voraciously during April and May, pupate during the latter part of May and the first part of June, and the beetles emerge during the latter part of June. The beetle, as with many *Rhyncophora*, is quite long-lived, and commonly lives until fall. It feeds upon the leaves, stems, and flower heads of clover, and causes a greater aggregate amount of damage than the larva stage. It does not usually begin to produce eggs until late summer, and some of the eggs hatching before cold weather produce the larvae, which may be found hibernating under rubbish in the clover fields, and which may even come out and feed during open periods in winter.

The larvae of *Phytonomus punctatus* feed chiefly at night,

and are to be found during the day on the ground, concealed under rubbish, or buried slightly in the loose soil. They are usually near a clover plant, but are sometimes found at quite a distance away; and they lie curled up, much as do the cut-worms or the saw-fly larvae.

The fully grown larva when extended is about 13 mm. long, mm. in diameter at the middle, and tapers gradually toward both ends. The head is brownish, and the general coloration is dark green, though the posterior half of the body is usually somewhat lighter and more yellowish, especially along the lateral margins; and along the median dorsal line is a broad, white, or pale rose-colored stripe, bordered on each side by a wide, interrupted black stripe, which is sometimes tinged with deep red.

There are no legs, but in their place are very prominent swellings or protuberances which have some resemblance to legs without claws. Those of the thoracic segments are conical, while those of the abdominal segments are somewhat transverse and each of them is longitudinally subdivided so as to form two lobes. The pro-thoracic segment bears three ventral protuberances, of which the middle one forms a prominent conical tubercle. This is divided at the tip to form two separate lobes, each with a stout, black, recurved bristle anteriorly, near its base. Similar but less conspicuous tubercles appear on each of the other thoracic segments, and each of the abdominal segments from 4 to 11 inclusive, bears a pair of the bilobed, slightly transverse protuberances described above. The posterior end of the body bears three rounded lobes which surround the anal orifice, one above, and two below.

Materials and Methods.

The method of building the cocoon was carefully observed in a large number of specimens. Many were carried in the pocket and kept under practically constant observation. Numerous contrivances were used to facilitate these observations, but the most satisfactory device consisted of a short piece of thin glass tubing, about one-half inch in diameter, into which a cylinder of white pith was closely fitted, the whole being enclosed in a second tube of black paper. Mature larvae were placed in small, oval cavities cut into the pith cylinder. These cavities were made about 6 mm. by 9 mm. at the surface, and a little less than 6 mm. deep. Thus, when the cylinder was inserted in the glass tube, the larvae were confined in cells that were similar in form and size to those normally made in the soil by this larva, except that a small portion of the complete oval was cut off on the open side by the inner surface of the glass. In spinning, the larva will attach the cocoon to the glass on the line where it intersects the oval, and will leave the window so formed unobscured until the cocoon is completed. The pith reflects the light through the meshes of the cocoon, and every detail of the movements of the larva can readily be observed.

For the anatomical and histological studies, larvae of different stages of development were used, including larvae killed when beginning to build. Unfortunately, material was not available, upon beginning these studies, for working out the course of the histolysis of the silk glands and the renewal of the Malpighian tubes in the adult, and such material could not be se-

cured in time to complete the studies in that direction. Repeated attempts were made to rear the larvae in the laboratory, some hundreds of larvae having been collected during the fall and winter for that purpose; but all were destroyed, apparently by a bacterial disease, before reaching maturity. It is necessary, therefore, to leave this part of the subject to be worked out later from timed specimens that are now being prepared.

The anatomy was studied both by dissection and by reconstruction from serial sections of the entire larva cut in both transverse and sagittal planes. Serial sections were also made from portions of the viscera that were dissected out in order to control more accurately the plane of the sections.

The histology of the silk-producing organs was also worked out from the serial sections just mentioned.

The material for dissection was killed in hot water at about 80 Centigrade. A part of this material was preserved in 80% alcohol and the remainder in a 5% solution of chloral hydrate.

In preparing the material for sectioning, the following fixing reagents were used:

1. Picro-Sulphuric Acid.

2. A saturated solution of Hg Cl in 35% alcohol ----98 parts
   Glacial acetic acid ------------------------------ 2 parts

Both reagents gave good results. Some of the larvae were killed by being put directly into the fixing solution heated to about 80 C. Others were killed in hot water and kept in water at 70 to 80 C. for five minutes before being placed in the fixing reagent. In all cases the body-cavity was opened to insure thorough penetration, after the fluids of the body had been coagulated by heat. After fixing, the reagent was washed out by repeated

changes of 70% alcohol and the specimens were finally preserved in 80% alcohol.

The usual paraffin method of embedding was employed, and serial sections 10 micra in thickness were cut with a Minot-Zimmerman Microtome.

For staining, Delafield's haematoxylin and Erlich's acid haematoxylin were used, differentiation being secured by over-staining and then decolorizing to the desired degree of intensity in alcohol slightly acidulated with hydrochloric acid.

Silk-spinning in General.

Silk is secreted by members of several classes of Arthropoda, and is employed by the animals producing it in building webs, nests, cases, cocoons, and supports of various kinds. In Arachnida, the spiders and pseudoscorpions produce this substance. The spiders use it in the construction of webs and in building their nests; and the eggs of many forms are enclosed in sacs composed of silk. The pseudoscorpions pass certain stages of their development enclosed within soft silken cases. Scolopendrella, which has been classed with Diplopoda, but which is regarded by some authorities as belonging to a distinct class, is also provided with silk glands, and the larvae of many very diverse orders of the Class Insecta produce silk.

Folsom has summarized silk production among insects as follows: "Silk glands, while most characteristic of Lepidoptera and Trichoptera, occur also in the cocoon spinning larvae of not a few Hymenoptera (saw flies, ichnenmons, wasps, bees, etc.), in Diptera (Cecidomyiidae), Neuroptera (Chrysopidae, Myrmelconidae), and in various larvae whose pupae are suspended from a silken support, as in the coleopterous families Coccinellidae and Chrysomelidae (in part) and the dipterous family Syrphidae, as well as most diurnal Lepidoptera." To the list of cocoon-building insects may be added the coleopterous genera Cionus and Phytonomus.

The silk glands of Lepidoptera and of Trichoptera have been studied and worked out in great detail by Helm, Blanc, Gilson, and others, and much work has been done upon these glands in Hymenoptera, chiefly in studies upon histolysis. These organs in

the other groups of insects seem to have received comparatively little attention, and I find no reference to the silk glands of Coleoptera.

Owing to its commercial importance and to its preeminence as a cocoon builder, the common silk-worm, Bombyx mori, has received more attention from students than any other silk-spinning insect. The details of the silk-producing organs of this insect, and its method of spinning have been very thoroughly worked out and are widely known. Therefore, in discussing these functions in another insect, it will be an advantage to compare them with those of the silk-worm, and to note their points of similarity and of contrast.

In common with the larvae of all Lepidoptera and of Trichoptera, the silk-worm is provided with a pair of silk glands which unite to form a single duct, the spinning tube, opening in the lower lip at the end of the lingula or hypopharynx; the latter being modified to form a spinneret. The glands are tubular and shining white; they are situated one on each side of the body; and are so long and convoluted as to envelop the posterior part of the alimentary canal. In each gland a spindle-shaped dilatation forms a silk reservoir which is connected by a slender tube with the common duct. The common duct, or spinning-tube, is a complex organ. After the separate, cylindrical streams of silk from the two reservoirs meet and unite upon entering the common canal at the posterior end of the tube, they pass into the muscular portion or "thread-press" where they are compressed and squeezed together and given the characteristic double, ribbon-like form of the thread. From here the thread passes on through the anterior portion or "directing tube" and, issuing from the

freely movable spinneret, in a semi-fluid condition is applied to the walls of the cocoon,where it quickly hardens. The cocoon is composed usually of a single, continuous thread, and the loops of this thread, while cemented together at the intersections by the gum, or "gres," with which the entire thread is coated, are visibly distinct. Furthermore, the gum may be softened in water at about 50 C. and the thread reeled off unbroken.

Cocoon Building of *Phytonomus punctatus*.

When the larva of *Phytonomus punctatus* reaches maturity it stops feeding, exhibits a strong negative reaction to light stimuli, and conceals itself, sometimes beneath rubbish, but more commonly by burrowing into the soil, where, at a depth varying from five or six to twenty millimeters, it forms an oval cell about nine millimeters long by six millimeters in diameter. After remaining quiescent for one or two days from the time it stops feeding, the larva begins the construction of its cocoon.

The cocoon is oval in form and averages 8 1/2 mm. in length by 6 mm. in diameter. Thecolor is pale yellow to nearly white when first built, but gradually changes to a rusty yellow or light brown. The walls of the cocoon consist of an irregular, coarse network of small, round or oval meshes. The separate threads composing the network become fused together and almost indistinguishable because of their plasticity when laid down and the abundant application of fluid silk from the interior after the network is complete. The fact that the finished cocoon is composed of threads at all is indicated only by the presence of a few free anchoring threads and by the sculpturing upon the outer surface.

The method of "spinning" seems to be unique among cocoon-building larvae, for I find no description of a similar habit in any other species. Even in regard to another species of the same genus, *Phytonomus* (*Hypera*) *rumicis*, J. A. Osborne# states simply that: "The spinneret of *Hypera rumicis* is anal; the silk

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issues in a very thick, clear, transparent thread, which eventually becomes reddish-brown." In all other methods of cocoon-building described in the available literature, the silk issues from either an oral or an anal spinneret terminating the silk producing organs, and is applied directly from this spinneret to form the walls of the cocoon.

With <u>Phytonomus punctatus</u> the silk substance issues from the anus. Upon resuming activity after the period of quiescence previously described, the larva ejects from the anus a rough stream of thick excrementitious matter mingled with silk substance. After a length of about five to eight millimeters this stream diminishes in diameter, and passes into a thread of clear, transparent silk. The larva now bends head to tail, applies the mouth-parts to the anal extremity, and fills the pharynx with the silk substance. During this operation the mandibles are widely separated and applied to the opposite sides of the anal segment, the act of filling the pharynx with silk substance being accompanied by constant, regular movements of the mouth-parts, and consuming three to five seconds.

In the construction of the cocoon, the silk is applied solely with the mouthparts. The thread issues between the mandibles and the labrum and is drawn out over the latter by the movement of the head in a ventral direction. The formation of the thread is accompanied by rapid movements of the mouth-parts, which are moistened by copious secretions from the salivary glands. When one mouthful of silk has been exhausted, the pharynx is refilled by applying the mouth-parts to the anus in the same manner as at first, and the process is repeated until the cocoon is complete. The silk issues from the mouth in a semi-fluid condition, and the

thread when first formed, is quite adhesive; but it quickly hardens upon exposure to the air.

In beginning to spin, the posterior part of the abdomen bends under and the larva braces itself by pressing two points of the dorsal surface of the body against opposite sides of the cell, leaving the head and anterior portion of the body free to move in any direction. The pharynx now being filled with the silk substance as described above, the head is bent as far back as possible and a drop of the clear, transparent, semi-fluid silk is forced between the mandibles and labrum and applied to the wall of the cell, to which it adheres. Now, with the mouth-parts moving rapidly, the larva carries the head in a ventral direction, drawing out a thread, which it attaches here and there to the walls of the cell. By repeatedly shifting the position of the body the thread is carried onward about the cell until the first mouthful of silk is exhausted.

The threads are then applied from fresh supplies of silk, and the cocoon is soon outlined by a network of very coarse meshes. The larva continues to apply new threads, reducing the size of the meshes until the cocoon assumes a regular, oval form. It then follows the threads with its mouth, strengthening them by additional applications of silk, and at the same time dividing the meshes that are still too large. This is continued until the cocoon is quite compact, with only small, round or oval holes through which the larva may be indistinctly seen. This application of fluid silk from the interior so fuses the threads together that the cocoon appears to be homogeneous, and the presence of threads in its structure appears, as previously stated, only in the anchoring threads and in the sculpturing of the surface.

After completing the cocoon the larva again becomes quiescent and lies coiled head to tail from six to ten days, when it moults and transforms into the pupa. The spinning of the cocoon is usually completed in from ten to fifteen hours.

The larva may spin its cocoon among the stems of a clover plant in a shaded cage, or when forced to do so can even build in a corner; but upon a flat surface it usually scatters the silk about uselessly and pupates without forming a cocoon. When not enclosedin a cell the larva first spins a number of short threads attached to the surfaces or objects within reach, which finally enclose it sufficiently to afford a support while outlining the oval cell. It then completes the cocoon as described above.

The average weight of the fully grown larva was found to be 0.0646 grams, and the average weight of three cocoons just spun under normal conditions, with the enclosed larvae was 0.0649 grams; the average weight of the enclosed larvae was 0.0415 grams and that of the cocoons was 0.0234 grams.

The cocoon of <u>Phytonomus punctatus</u> as seen from the above description is not composed of a single continuous thread, as is the cocoon of the silk worm. Instead, it is composed of many comparatively short threads, ten to fifteen or sixteen millimeters long, and of silk substance that is spread, not spun, along the threads composing the network in strengthening them. The threads made by Phytonomus vary in diameter, each span usually being thickest at the points of attachment and tapering from both ends toward the middle. This is due to the fact that the larva seems habitually to apply tractive force to the thread just before attaching it, and to force out a fresh droplet of

silk in making each connection. The diameter of the threads at their middle varies from 0.03 mm. to 0.08 mm.; at the points of attachment, however, they are not cylindrical and vary greatly in form and size.

As a contrast with the short, uneven threads spun by *Phytonomus punctatus*, the continuous thread which the silk-worm spins in forming its cocoon is stated by various authorities to measure from 800 to 1500 meters in length. This thread is thickest in the middle and tapers gradually to both ends. In a series of elaborate measurements made in 1885, by T. Wardle, the double thread or "bave" as spun by the silk-worm measures on the average, 0.031 mm. to 0.036 mm. at the thickest part, and 0.028 mm. to 0.025 mm. at the thinnest part; and in some instances the middle part was found to be one third thicker, stronger and more elastic than the ends.

Malpighian Tubes in Phytonomus.

The difference between the silk-producing organs of *Phytonomus punctatus* and those of the common silk-worm is as wide as the difference between their methods of spinning. The silk-worm is provided with a pair of specific larval glands, the only function of which is to produce the silk for the protecting cocoon of the pupa stage. The larva of *Phytonomus punctatus*, however, while it builds a relatively heavier cocoon, has no such narrowly specialized organs.

Here occurs the most remarkable condition encountered in the course of these studies. A very unusual alternation of glandular functions appears in the fact that in this larva the Malpighian tubes become greatly enlarged, in the fourth instar, and function as silk glands at the time of spinning.

The larva of *Phytonomus punctatus* has three pairs of simple Malpighian tubes. These organs are slender, blind, tubular glands which arise from the anterior end of the proctodeum. Each of these Malpighian tubes consists of three distinct divisions, viz., a duct or "ureter," a glandular secreting portion about six times as long as the duct, and a very slender attenuated portion at the blind end, which is nearly as long as the duct.

The Malpighian tubes arise in the same transverse plane from a distinct enlargement at the anterior end of the ileum, the ducts are all directed backward at the point where they enter the intestines, and form narrowly-acute angles with the sides of the mesenteron, which is as narrow here as the ileum. The ducts are closed by membranous folds within the lumen of the intestine,

the valves thus formed appearing much as if the tubes had been cut off squarely and then inserted obliquely through the wall of the intestine thus:

These valves are so formed that they permit the secretions of the glands to pass freely into the lumen of the ileum, but close by pressure from within the latter so as to prevent the contents of the intestines from passing backward into the ducts.

The general disposition of the Malpighian tubes within the body cavity is quite constant. As previously stated, the ducts at first all run in an anterior direction from the point of attachment. Two pairs continue in this direction as far as the third thoracic segment, running along the ventral floor of the body cavity, one pair on each side of the central nervous system and with the ducts of each pair lying closely parallel to each other. Each of these four ducts passes into the secreting portion of the Malpighian tube near the third thoracic segment; the point of connection between the two portions being rendered quite distinct in the older larvae by the abrupt change from the narrow, comparatively smooth duct to the larger, strongly lobulated glandular part. From here, each gland forms an arch, and returns in a posterior direction repeatedly folded upon itself in the plane of the thin fat-body surrounding the large stomach. These tubes are embedded in the fat-body, as indicated, and the

glands of each pair form approximately parallel, longitudinal bands reaching to the posterior end of the stomach; the stomach being nearly surrounded by the combined width of these bands.

The other two ducts run in an anterior direction from 1/4 to 1/2 mm. and then, doubling sharply upon themselves, run parallel down the sides of the ileum, diverging, and passing into the glandular portion near its posterior end. The glandular parts of this pair of tubes are strongly convoluted, and with the fat-body in which they are embedded, fill the posterior part of the body-cavity and enclose the hinder parts of the alimentary canal.

The glandular portions of all six of the Malpighian tubes converge toward the anterior end of the colon, where they pass into the slender distal portion. The surface of the colon is divided by longitudinal lines into six distinct areas; and the slender distal portion of each of the tubes mentioned above, passing under the peritoneal membrane which encloses colon, at the anterior end of the latter, enters one of these areas. Each tube now takes a sinuous course along the area to which it belongs, and all terminate near the junction of the colon with the rectum.

The Malpighian tubes are silvery white, and in the young larva there is little in the general appearance of the tubes to distinguish the secreting portion from the duct, except the more pronounced lobulation of the former. These portions of the gland are nearly equal in diameter, and the difference in the character of their surfaces is due to the fact that in a transverse section of the secreting portion, there are only two to five large cells, while a similar section through the walls of the duct shows five to seven smaller cells. As the larva approaches maturity the secreting cells increase in size and the lobulation becomes more

strongly marked.

In the mature larva, just before the Malpighian tubes change their function, these glands are about 3 1/2 times the length of the body; the duct is about 5 1/2 mm. long, the glandular portion 35 mm. long, and the slender distal portion 5 mm. long.

The glandular portion of the tube is not linear, because of the lobulation; but its average diameter is about 0.35 mm., that of the duct is 0.1 mm., and that of the distal portion 0.02 mm. The length of the proctodaeum from the point at which the Malpighian tubes arise to the anal extremity, including the ileum, colon, and rectum, is 11.5 mm.

In Phytonomus, the proctodaeum performs the same function as the silk reservoirs of Bombyx mori, and since these are included in the published length of the silk glands of that and related insects, it becomes difficult to make a satisfactory comparison between the length of the silk producing organs in Lepidoptera with the length of the analogous organs in Phytonomus. In making such a comparison, I shall take for the lepidopterous species, the total length of both glands (twice the length given by Helm and by Packard) and for Phytonomus, six times the length of a single Malpighian tube plus the length of the proctodaeum from the insertion of the Malpighian tubes to the anal extremity. The ducts of the Malpighian tubes will fairly balance the non-secreting ducts of the silk glands, but it is a question if it would not be fairer to take the length of the proctodaeum of Phytonomus six times rather than once, in order to offset the ratio of the true silk reservoirs to the secreting portion of the glands of Lepidoptera.

In the following table the first four species are Eurasian ceterpillars, and the data is quoted by Packard from Helm. Telea polyphemus was added to the list by Packard himself.

	Length of Larva	Length of Silk Glands	Total Length of Silk Producing System	Ratio of Total Length of Silk Producing System to Length of Body.
Vanessa io	32 mm.	26 mm.	56 mm.	1.75
Smerinthus tiliae	63 mm.	205 mm.	410 mm.	6.50
Bombyx mori	56 mm.	262 mm.	524 mm.	9.36
Antheraea yamamaya	100 mm.	625 mm.	1250 mm.	12.50
Telea polyphemus	60 mm.	900 mm.	900 mm.	15.00

Phytonomus punctatus	13 mm.		284.5 mm.	21.88

It can be seen from this table that the relative total length of the silk producing system in Phytonomus punctatus is approximately 1 1/2 times greater than that of Telea polyphemus, and 2 1/3 times greater than that of Bombyx mori.

Histology of the Malpighian Tubes

In Phytonomus punctatus, the Malpighian tubes with the fat body and other organs are surrounded and bound together by nucleated peritoneal membranes or strands of connective tissue. A transverse section of a Malpighian tube, therefore, shows first a thin, nucleated peritoneal membrane, and then, a very delicate, apparently homogeneous basal membrane; within this is a single layer of polygonal cells which project strongly into the lumen of the tube. Within the latter is a very thin membrane, or intima, which is as delicate as the basal membrane. The highest magnification available (a 1/12 inch oil immersion objective plus a 3/4 inch ocular) showed no transverse striations of this membrane, which appears to be nothing more than the normal cell wall.

The basal portion of a Malpighian tube has been termed a duct in this paper, because it serves as such while silk is being secreted. In the very young larva the entire tube from the point of insertion to where it passes under the peritoneal sheath of the colon, is quite uniform in structure and appearance. Its walls are composed throughout, of small cells with large round or oval nuclei, and the cells being set more closely together, the middle portion of the tube does not show the differentiation that is shortly to appear.

Very soon, the cells of the middle three quarters of the tube begin to increase in size, causing the tube to become longer and giving to it the lobulated appearance that is characteristic of this portion in all of the later stages. As this increase in

the size of the cells continues, the nuclei lose their oval form and become branched. When the larva is approaching maturity the diameter of the middle section of the tube, as previously stated, becomes about four times that of the basal section. The cells project into the lumen of the tube so as almost completely to close it. The nuclei assume a position near the wall of the lumen, toward which they present a smoothly rounded surface; but from the opposite side numerous, diffuse, sometimes fantastically forked branches radiate through the cytoplasm toward the peripheral side of the cell. When the larva is nearly mature the cytoplasm of these cells presents a perfectly uniform alveolation and appears to have ceased from active secretion. The complete absence of vacuoles seems to indicate that these cells have now lost their original, renal, excretory function.

While these changes have been taking place in the cells of the middle sections of the Malpighian tubes, the cells of the basal sections or "ducts" have also been growing; but only in keeping pace with the growth of the larva. They retain their relative size and position in relation to the walls of the tube, and the nuclei of these cells remain oval in form throughout the history of the organs. These cells also project into the lumen of the tube to such a degree that it is nearly closed. When the larva is fully developed, and the cells of the middle section of each Malpighian tube have become inactive the cells of the basal portion are seen still to be actively secreting. Numerous elongate or crescent-shaped vacuoles which appear to be filled with a clear secretion occur in the cytoplasm near the peripheral wall of the cells, and smaller round or oval vacuoles are distributed through the cytoplasm up to the walls of the lumen.

The short basal portion is probably sufficient to provide for excretion during the inactive period preceding the building of the cocoon, during which the cells of the middle section are becoming prepared for activity in their new function of silk secretion. The fact that a portion of the organ retains the original characteristics of the Malpighian tubes, occurs, probably as an adaptation to provide for the continuance of excretion up to the time of spinning; for there is no other apparent reason for the long ducts into which the basal portion of the tubes is converted during the process of silk production.

Silk Production by Phytonomus punctatus.

In the sections of the preserved material, the silk substance first appears as minute granulations in the cytoplasm which are about twice to three times the diameter of the alveoli of which the cytoplasm is composed. These granulations accumulate to form masses or vacuoles in which the secretion retains its granular appearance. During the secretion of the silk, the nuclei of the secreting cells lose their sharply defined branches and clear outlines, and become somewhat diffuse at the margin; but the body of the nucleus takes a deeper stain than before secretion begins. The intima disappears, and the vacuoles become connected with the lumen by sinuses which widen until the vacuole and the lumen are confluent. As the process of secretion goes on, the nuclei appear more and more diffuse, the cytoplasm of the cells diminishes in quantity, the lumen of the tube becomes distended with the granular silk substance, and its walls, in longitudinal section, present a very uneven outline.

As a duct, the basal portion of the tube becomes distended with the silk substance to form a thin-walled canal; the cells with their nuclei becoming quite flattened, and no longer showing any evidence of activity. This duct conducts the silk substance to the proctodaeum where it accumulates in the colon and rectum to be drawn upon as described under the head of cocoon spinning.

During the period of quiescence which precedes the building of the cocoon, the regeneration of the alimentary tract begins. Cellular activity appears first at the anterior and posterior extremities of the mesenter~~am~~on, in the regions of the oesophageal

and the pyloric valvules, and when the secretion of silk begins, both the posterior opening of the oesophagus with a considerable portion of its length, and the anterior opening of the proctodaeum are completely closed by plugs of newly formed tissue. The presence of these obstructions, first, compels the exit of the silk substance from the proctodaeum through the anus only; and, second, prevents the contents of the pharynx from passing backward through the oesophagus.

Locomotion.

As stated in one of the preceding sections, the larva of Phytonomus punctatus is legless. It is, however, quite active in ascending the clover plants to feed upon the leaves, and in descending to secure concealment from too intense daylight. It is able, also, to move freely over the ground, and even to travel readily upon a smooth glass surface. If there is a film of moisture upon the glass, the larva can readily make its way up a vertical surface, and a young larva can even travel quite freely upon the under surface of a horizontal plate of glass.

In speaking of the habits of the larva of Phytonomus punctatus, C. V. Riley# says: "When teased, they finally stretch out and walk off more rapidly than could be expected of a legless Curculionid larva. When crawling, they not only use the ventral tubercles, which are very prominent, resembling legs without the claws, but they use also the head and anus in a very peculiar manner. The head is pressed downward until the front touches the ground. The body is thus stretched forward as much as possible, when the anus leaves its hold, quickly following the rest of the body and taking a firm hold near the head. The larva then stretches itself out, and the same movements are repeated. The anus evidently plays an important part in the locomotion; it is somewhat extensile, and each time the larva uses it to take hold of a leaf, a small drop of a sticky fluid is ejected. The anus seems also to possess the power of suction, as the larvae are capable of erecting themselves, so as to look around for some object to take hold of; turning, at the same time, their

Ann. Rept. U.S.Dept.Agr., 1881 (1882).

bodies in all directions and holding solely by the anal end."

This larva does not normally emit a sticky fluid from the anus to aid in locomotion. I have carefully tested this on clean glass slides, after causing the larva first to crawl over a dry cloth, in order to remove any moisture that might be adhering to the body, and I find that the larva does not leave a trace of fluid upon the glass. A film of moisture undoubtedly aids the larva in moving upon a perfectly smooth surface; and when it is upon such a surface and discharges its excrement, some of the fluids are drawn under and around the body by capillarity. These fluids remain for some time without drying, and when not removed, do mark the trail of the larva as it moves.

In some larvae, as those of Lampyridae, the anus can be everted to form a sucking organ, and is used in locomotion. In the larva of Phytonomus punctatus, however, this is not the case. In this larva, the ventral portion of the posterior segment is so formed that it can be used as a sucking organ when the larva is on a smooth surface. This organ is so effective that the larva is able to erect itself with only this organ supporting it, and to swing the body from side to side in searching for a suitable place to attach the anterior end.

The posterior segment bears two protuberances on the ventral portion of the anterior half, and the posterior half, with the anus, may be retracted or extended. When on a smooth surface, as a glass slide, the anus is extended and the two protuberances just mentioned, the two lobes on the ventral side of the anal orifice and the ventral surface of the segment between these points, are applied to the glass, upon which they flatten out so as to bring all parts of the surface in contact with the glass.

Two sets of lateral segmental muscles converge and are inserted upon the ventral body wall close to the median line and near the center of this surface. These muscles raise the center of the area that is pressed against the glass, forming a vacuum which easily supports the weight of a larva of six millimeters length from the under surface of a horizontal glass slide.

This sucking organ is naturally more effective upon a moist surface, and it is habitually released by retracting the extended portion of the anal segment and thus rolling the attached surface away from the glass from the rear, until the center is reached. The pair of ventral protuberances of the anterior half of the posterior segment also serve as grasping organs somewhat analogous to anal pro-legs when the posterior half of the segment is retracted.

The ventral prominences of the first thoracic segment may also serve in locomotion as a feeble sucking organ, but it will not support the weight of the larva except when very young, and then, only from a moistened surface.

The larva evidently uses the mouth-parts in crawling, in preference to the thoracic lobes, since it habitually gropes about, moving the mandibles as if searching for something to sieze, before applying the ventral parts of the thorax to the surface. When placed on a hairy leaf it extends the body, siezes

two or three hairs with the mandibles, and without using the thoracic lobes, drags the body forward, by turning the head far under the thorax. The posterior end of the body is now drawn forward and attached, the body again stretched ~~attached~~, and the process repeated.

In ascending the smooth stem of white clover, the larva coils about the stem, attaches the posterior sucker, and swings the anterior end of the body upward. Then applying the thoracic sucker to the stem and supplementing it by gripping the stem between the head and posterior segments of the thorax, it quickly swings the hinder end of the body upward and secures a new grip with the posterior sucker. By repeating these operations it makes surprisingly rapid progress for a larva with locomotor organs that are so little developed. Upon the hairy stem of red clover, the progress of the larva is aided by its catching the hairs with the mandibles and by grasping them between the folds of the body and between the ventral protuberances.

Summary

Reviewing briefly the observations upon silk production and cocoon building by Phytonomus punctatus that are presented in this paper, we find the following interesting facts.

The larva of this beetle builds a cocoon which is relatively as heavy as that of many of the principal cocoon-building Lepidoptera.

It has no special silk glands; the silk being secreted by the cells of a certain definite portion of each of the Malpighian tubes which lose their renal function some time before beginning to secrete the silk.

The basal ninth of each Malpighian tube retains its renal function during the interval between cessation of that function by the silk secreting cells and the commencement of silk production; these basal portions serving later as ducts which conduct the silk substance to the ileum.

Regeneration of the alimentary tract begins during the interval of quiescence preceding the building of the cocoon; cellular activity appearing first in the regions of the oesophageal and the pyloric valvules, completely closing both the posterior end of the oesophagus and the pyloric end of the ileum by plugs of new tissue before silk production begins.

The proctodaeum serves as a silk reservoir from which, owing to the closure of its anterior end as mentioned in the preceding paragraph, the silk can issue only at the anus.

The larva builds the cocoon by repeatedly filling the pharynx with silk substance from the anus and forming it into threads with the mouthparts.